Design, synthesis, and evaluation of novel quinoline containing compounds as anticancer agents

Introduction: Quinoline is a heterocyclic aromatic compound found in a diverse range of natural and pharmacologically active molecules, exhibiting a wide array of biological activities, including anticancer activity. Furthermore, vascular endothelial growth factor receptor-2 (VEGFR-2) is a crucial receptor tyrosine kinase that plays a vital role in the proliferation, invasion and migration of endothelial cells, thus influencing cell survival, vascular permeability and neovascularization. Studies have shown that VEGFR-2 is expressed extensively in various types of cancers. As a result, VEGFR-2 inhibition has been accounted as a strategic target for antitumor therapy.

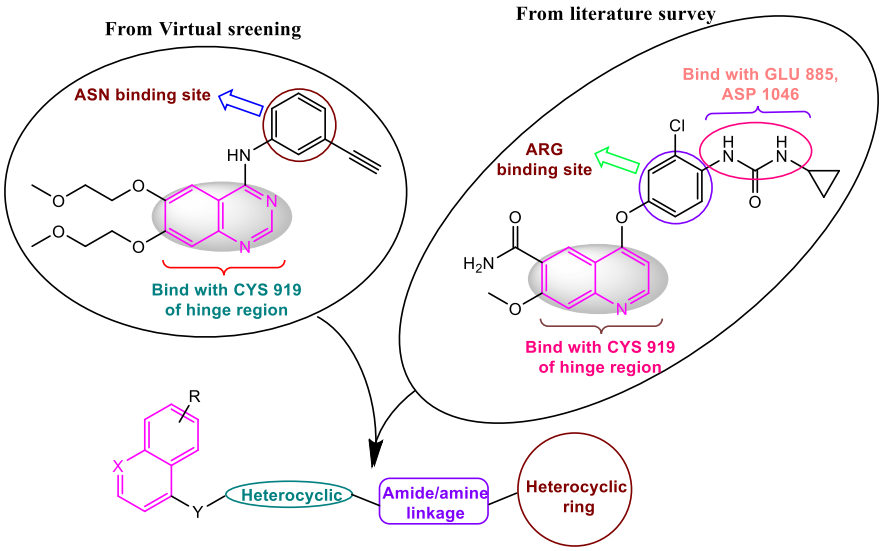
Owing to the significance of the quinoline nucleus as an anticancer agent and the involvement of VEGFR-2 in cancer progression, the designed novel quinoline analogues could potentially act against VEGFR-2, offering a promising new treatment against cancer.

Objectives:

1) Exploring the potential of VEGFR-2 inhibitors in Cancer treatment.
2) To carry out *in-silico* study for the designing and identification of VEGFR-2 inhibitors.
3) Synthesis of designed analogues and their characterization.
4) To perform the biological activity of the synthesized compounds.

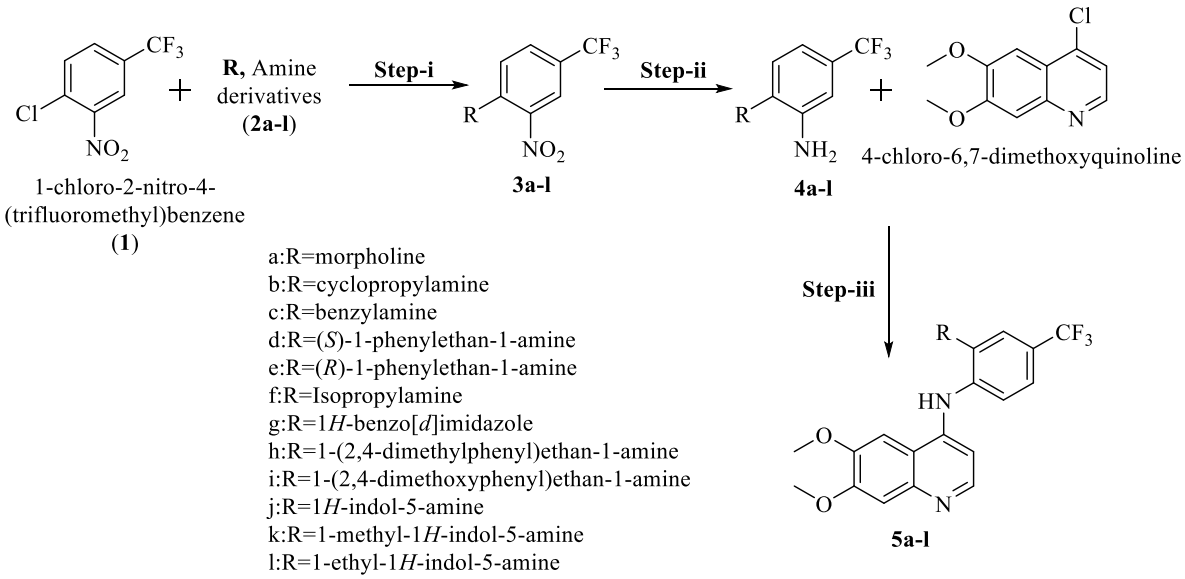
Methodology (work done):

Designing: The synthesis schemes were designed based on the pharmacophore obtained through virtual screening of different databases (USFDA approved drugs, USFDA withdrawn drugs, Maybridge, MDPI, Speck database) and literature survey.

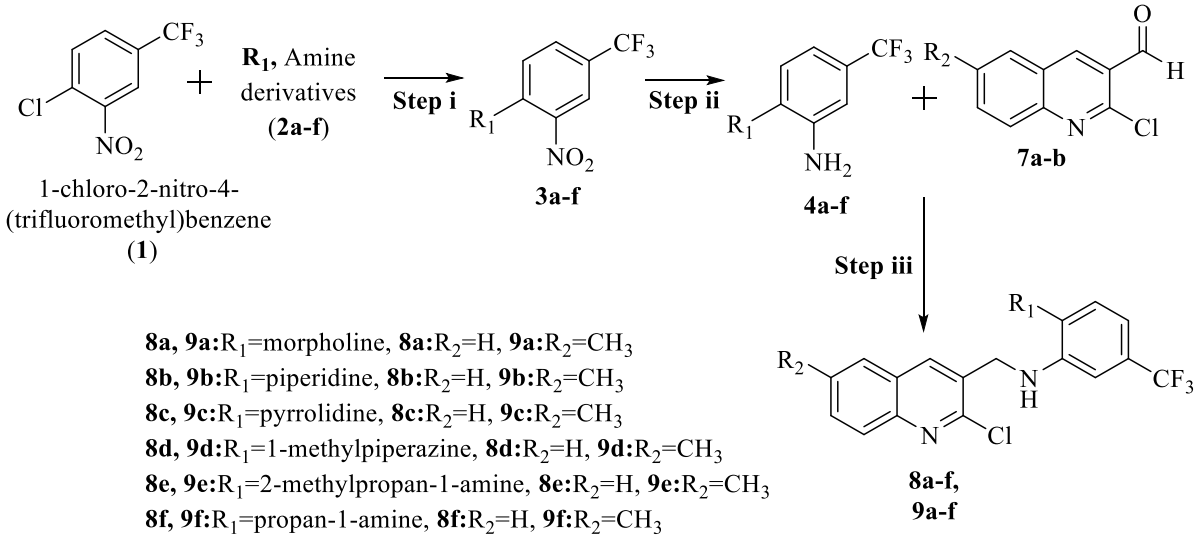


Synthetic study: Several synthetic schemes were designed, but only three were successfully implemented to synthesize the desired compounds. The reactions were monitored on silica TLC plates to ensure completion of the starting material, and the purification was achieved using 60-400 mesh size silica-gel column chromatography to obtain the desired products.

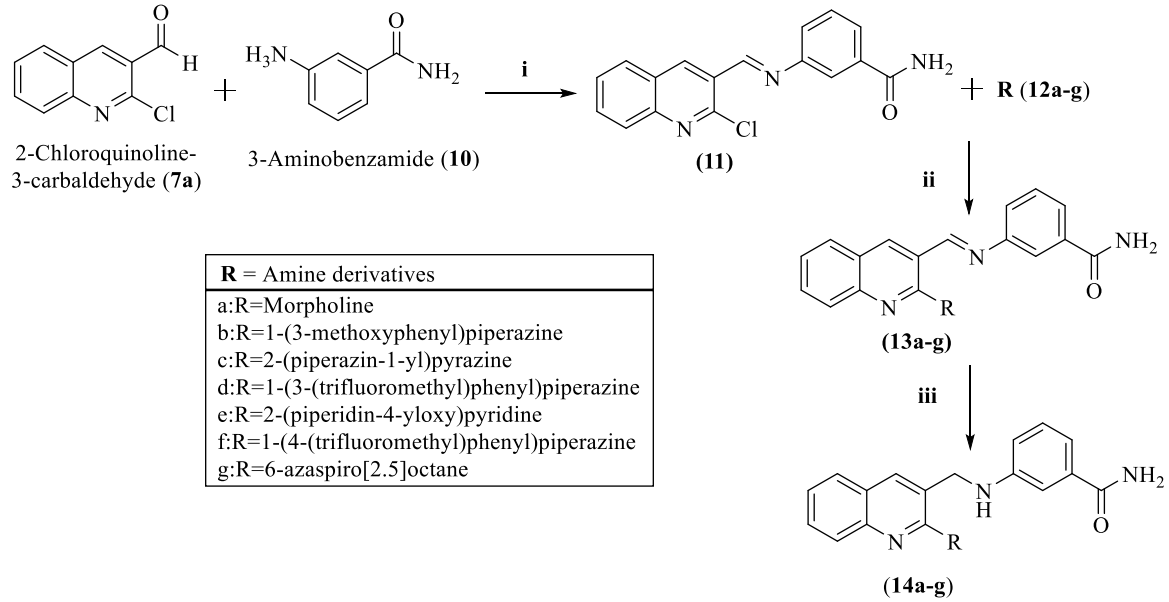
Scheme-1



Scheme-2



Scheme-3



R = Amine derivatives
a:R=Morpholine
b:R=1-(3-methoxyphenyl)piperazine
c:R=2-(piperazin-1-yl)pyrazine
d:R=1-(3-(trifluoromethyl)phenyl)piperazine
e:R=2-(piperidin-4-yloxy)pyridine
f:R=1-(4-(trifluoromethyl)phenyl)piperazine
g:R=6-azaspiro[2.5]octane

Structural elucidation: The final synthesized compounds were analyzed using 1H-NMR spectroscopy and further confirmed by mass spectrometry.

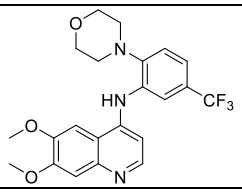
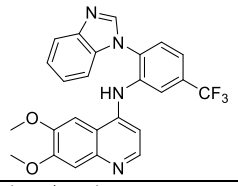
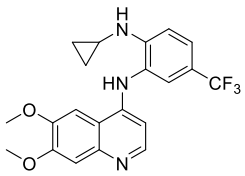
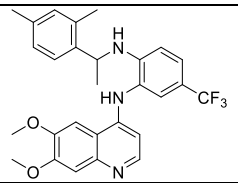
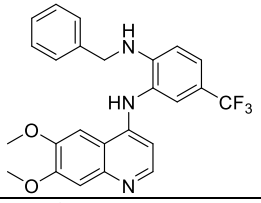
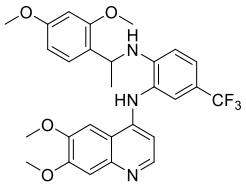
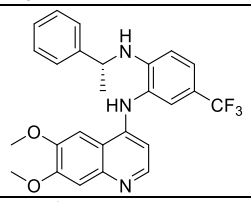
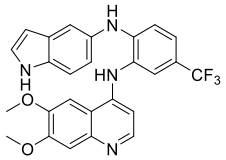
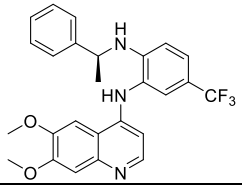
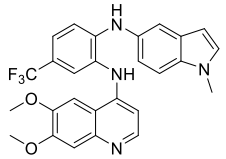
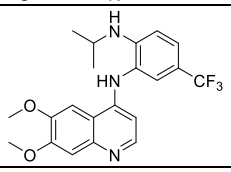
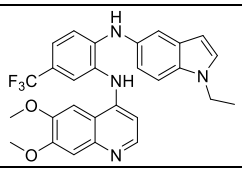
Biological evaluation: *Invitro* biological activity was evaluated using Kinase-Glo MAX Luminescence assay kit from BPS Bioscience (Catalog number: 40325), according to the prescribed protocol.

Results:

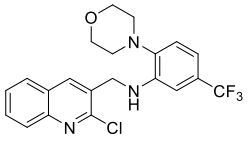
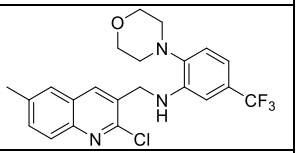
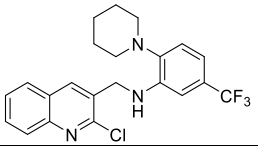
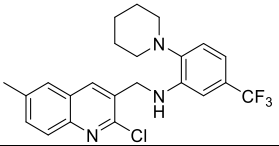
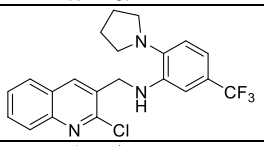
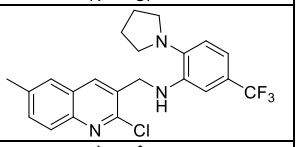
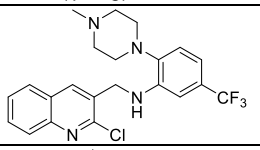
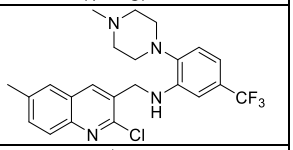
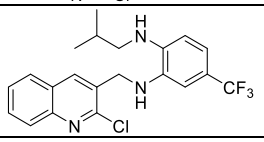
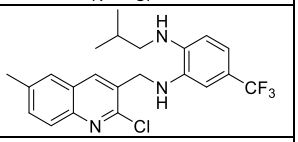
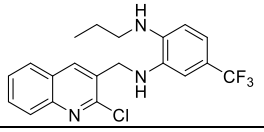
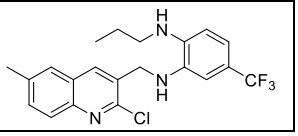
Designing: Synthesis schemes were designed based on the quinoline pharmacophore obtained from virtual screening and literature survey, possessing bulky substituents at 3rd or 4th position and an electron donating group at either the 6th or 7th position or in both positions.

Synthetic study: Two synthetic schemes were successfully employed to synthesized 19 quinoline derivatives. Among them, 12 were 4,6,7-substituted, 3 were 2,3,6- substituted and 4 were 2,3-substituted quinoline derivatives. Both synthetic strategies consist of three to four steps: Cl-displacement, reduction, Buckwald reaction and reductive amination. The synthesized quinoline derivatives were obtained as semisolid or solid, with percent yields ranging from 25% to 75%.

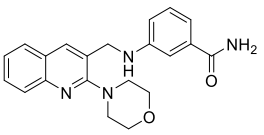
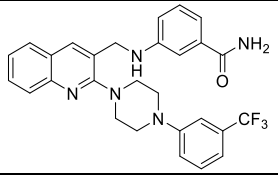
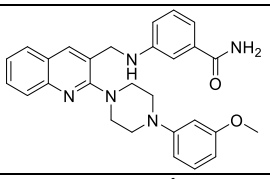
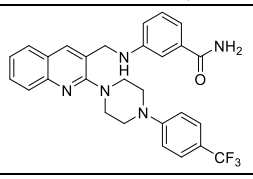
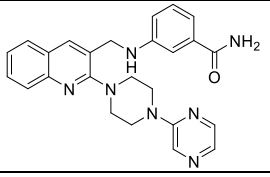
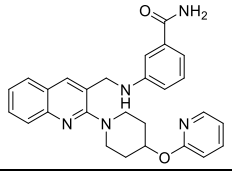
First series of Compounds (Scheme-1)

S.No	Product Code	Chemical Structure	S.No	Product Code	Chemical Structure
1	**6a**		7	**6g**	
2	**6b**		8	**6h**	
3	**6c**		9	**6i**	
4	**6d**		10	**6j**	
5	**6e**		11	**6k**	
6	**6f**		12	**6l**	

Second series of Compounds (Scheme-2)

S.No	Product Code	Chemical Structure	S.No	Product Code	Chemical Structure
1	**8a**		7	**9a**	
2	**8b**		8	**9b**	
3	**8c**		9	**9c**	
4	**8d**		10	**9d**	
5	**8e**		11	**9e**	
6	**8f**		12	**9f**	

Third series of Compounds (Scheme-3)

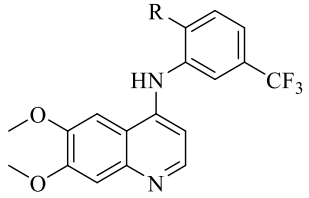
S.No	Product Code	Chemical Structure	S.No	Product Code	Chemical Structure
1	**14a**		4	**14d**	
2	**14b**		5	**14e**	
3	**14c**		6	**14f**	

Structural elucidation: All the synthesized quinoline derivatives were confirmed by identification of their characteristic peaks in the 1H-NMR spectroscopy, which is further

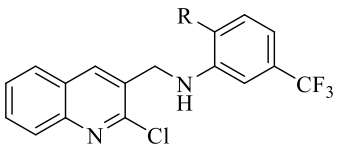
confirmed by mass spectrometry.

Molecular docking study: All the synthesized compounds (**5a-l, 8a-f, 9a-f** and **14a-g**) were subjected for molecular docking study using GLIDE tool against the targeted protein (VEGFR-2, PDB ID: 6GQO). To comprehend the interaction pattern between ligands and proteins. All the compounds exhibited potent to moderately favourable interactions and docking scores. The docking scores of the compounds ranged from -8.045 to -3.096 kcal/mol. Compound **5i** displayed the highest docking score (-8.045 kcal/mol), while the reference drug Lenvatinib showed a docking score of -11.066 kcal/mol.

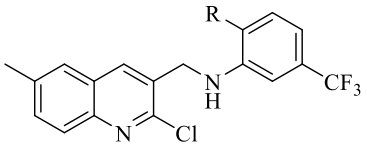
Table 1. Docking results of all the synthesized compounds.



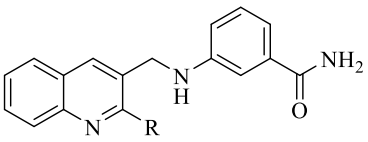
5a-l



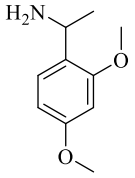
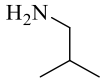
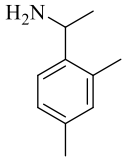
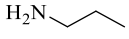
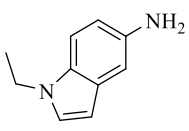
8a-f

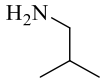
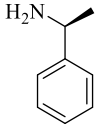
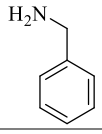
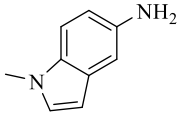
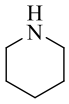
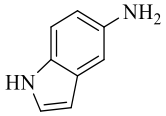
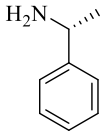
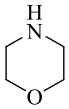
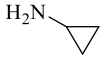
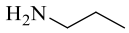
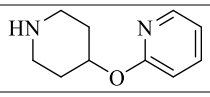
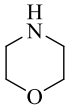
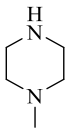
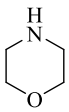


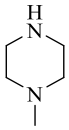
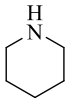
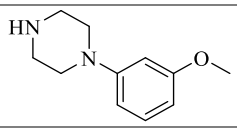
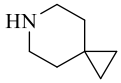
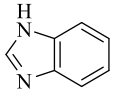
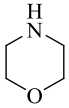
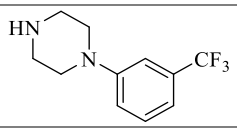
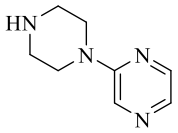
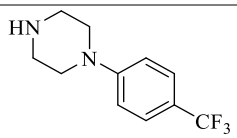
9a-f



14a-g

Cmpd.	R	Docking score (kcal/mol)	H-bond interactions (Distance)	Other interactions
5i		-8.045	GLU917 (2.79Å), ASP1052 (2.79Å)	**Aromatic H-bond** LEU840, CYS919 **π-π interaction** PHE1047
8e		-8.011	GLU885 (2.06Å)	**Aromatic H-bond** ASP1046
5h		-7.974	None	**Aromatic H-bond** GLU850, CYS919
8f		-7.873	ASP1046 (2.28Å), GLU885 (2.24Å)	**Aromatic H-bond** ASP1046 **π-π interaction** PHE1047, LYS868
5l		-7.673	GLU885 (2.09Å)	**Salt bridge** ASP1046 **Aromatic H-bond** ILE1044, GLU885, ASP1046, VAL914 **π-π interaction** LYS868

9e		-7.573	CYS919 (1.93Å)	None
5d		-7.484	None	**Aromatic H-bond** ILE1044 **π-π interaction** HIS1026, TYR1082
5c		-7.419	GLU885 (1.62Å &1.71Å)	**Aromatic H-bond** LYS868, ASP1046
5k		-7.331	GLU885 (1.81Å &2.52Å), LYS868 (2.64Å)	**Aromatic H-bond** GLU885 **π-π interaction** LYS868
8b		-7.323	ASP1046 (2.28Å)	**Aromatic H-bond** GLU885 **π-π interaction** LYS868
5j		-7.217	GLU885 (1.82Å &2.55Å), LYS868 (2.63Å)	**Aromatic H-bond** GLU885 **π-π interaction** LYS868
5e		-7.088	GLU885 (2.46Å), ASP1046 (2.08Å), LYS868 (2.38Å)	**Salt bridges** ASP1046 **Aromatic H-bond** ASP1046 **π-π interaction** PHE1047
8a		-7.048	ASP1046 (2.01Å)	**Aromatic H-bond** GLU885, ASP1046 **π-π interaction** LYS868, PHE 1047
5b		-6.784	GLU885 (1.90Å)	**Salt bridge** ASP1046
8c		-6.68	ASP1046 (1.96Å)	**Aromatic H-bond** GLU885 **π-π interaction** LYS868, PHE 1047
9f		-6.619	None	**Aromatic H-bond** GLU885, ASP1046 **π-π interaction** LYS868
14e		-6.45	CYS919 (2.50Å), LEU 840 (1.97Å)	**Aromatic H-bond** LYS920, ASP1046
14a		-6.359	CYS919 (2.48Å), ALA 1050 (2.72Å)	**Aromatic H-bond** CYS919
9d		-6.309	None	**Aromatic H-bond** ASP1046, ASP814, ILE1025
9a		-5.783	None	**Halogen bond** ASP1046 **Aromatic H-bond** HIS1026

9c		-5.708	None	**Halogen bond** ASP1046 **Aromatic H-bond** HIS1026
8d		-5.448	None	**Halogen bond** ASP1046 **Aromatic H-bond** HIS1026
9b		-5.582	None	**Halogen bond** ASP1046 **Aromatic H-bond** ILE1025 **π-π interaction** HIS1026
14b		-5.395	LEU840 (2.17Å), ALA 1050 (1.85Å)	**π-π interaction** PHE1047
14g		-5.339	LEU840 (1.79Å)	**Aromatic H-bond** LEU840, CYC919 **π-π interaction** PHE1047
5f		-5.269	ASP1046 (2.36Å)	**Salt bridge** ASP1046, GLU885
5g		-5.075	CYS919 (2.00Å)	**Aromatic H-bond** PHE1047, CYC919
5a		-4.579	None	**Salt bridge** ASP1046, GLU885 **Aromatic H-bond** GLU885
14d		-4.441	ASP1046 (2.09Å), ARG1027 (2.27Å), TYR1082 (2.36Å)	**Aromatic H-bond** ASP1046
14c		-4.125	LEU840 (2.31Å), ARG1032 (1.96Å), ARG1051 (1.91Å & 2.24Å)	**Aromatic H-bond** LEU840 **π-π interaction** PHE1047 LYS838
14f		-3.096	None	**π-π interaction** LYS838
Lenvatinib (standard)	----	-11.066	CYS919 (2.10Å), ASP1046 (2.16Å), GLU885 (1.98Å &2.39Å)	**Aromatic H-bond** CYS919, GLU917, PHE1047

Biological evaluation:

VEGFR-2 enzyme inhibition study: All synthesized compounds (**5a-l, 8a-f, 9a-f** and **14a-g**) were screened using molecular docking study before subjected to the *in vitro* VEGFR-2 enzyme inhibition study. Following the docking studies, 12 compounds were selected for

the study, with at least one compound included from each series. Synthesized compounds were assessed for their inhibitory activity against VEGFR-2, using Lenvatinib as a reference drug. All the compounds exhibited potent to moderate activity, with IC_{50} ranging from 321 nM - 1.2 µM.

Table 2. The results of VEGFR-2 inhibitory activity of the synthesized compounds and are represented as IC_{50} in nM.

S.No	Compound	IC_{50} (nM)	Degree of Activity
1	5i	321 ± 11.3	
2	5k	406 ± 29.1	Highly active
3	5a	445 ± 23.4	
4	5h	567 ± 10	
5	9e	632 ± 8.5	
6	8e	762 ± 11.9	Moderately active
7	8f	775 ± 8.3	
8	5l	883 ± 14.1	
9	5d	1008 ± 10.4	
10	5c	1069 ± 12.8	Less active
11	8b	1276± 31.2	
12	14e	1289± 14.7	
13	Lenvatinib	415 ± 16.6	

Cytotoxicity study: Among all the synthesized compounds, those with activity comparable to the reference drugs in the enzyme inhibition study (**5a**, **5i** and **5k**) were selected for *in vitro* anticancer activity testing against the HepG2 and HUVEC cell lines, using Lenvatinib as a reference drug. The result of the study was shown in the table no 3.

Table 6.4. Results of cytotoxic activity screened against HepG2 cell line.

Compounds	IC_{50} (µM)	Activity
5i	18.11 ± 2.76	Potent
5k	34.46 ± 7.17	Moderately potent
5a	59.27 ± 1.83	Less potent
Lenvatinib	26.95 ± 4.81	

Conclusions:

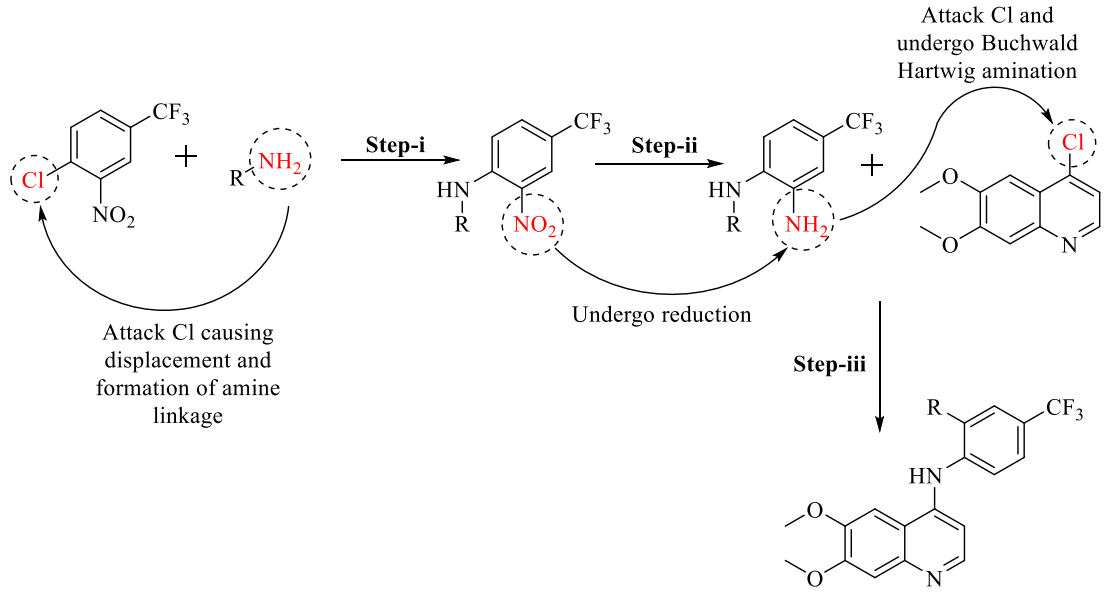
The current research yielded the following significant findings and conclusion:

- In the present study, the important structural features required for VEGFR-2 inhibition were identified through structure-based virtual screening of public domain databases (USFDA approved drugs, USFDA withdrawn drugs, Maybridge, MDPI, and Speck database) and a literature review.
- Based on the identified structural features, compounds and synthetic schemes were designed. A total of 31 new quinoline-based derivatives were synthesized from three synthetic schemes and divided into four series. The newly prepared derivatives were characterized by appropriate spectral techniques (NMR, MS and FT-IR) and are in agreement with the chemical structure of compounds. The physical properties such as melting point, R_f values were measured for all the synthesized compounds.
- The interactions of all synthesized compounds with the target receptor were investigated at the atomic level in the molecular docking. Based on docking study, 12 compounds were selected enzyme inhibition study, with at least one compound included from each series. The selection was determined by the highest docking score obtained from each series of compounds. Compounds **5a, 5c, 5d, 5h, 5i, 5k, 5l** (series-1), **8b, 8e, 8f** (series-2), **9e** (series-3) and **14e** (series-4) were selected. The results of the enzyme inhibition study revealed that commendable anti-VEGFR-2 potential was exhibited by some of the selected compounds. Compound **5i**, **5k** and **5a** (IC_{50} = 321, 406 and 445 nM, respectively) displayed VEGFR-2 inhibitory activity comparable to the reference drug Lenvatinib (IC_{50} = 415 nM).

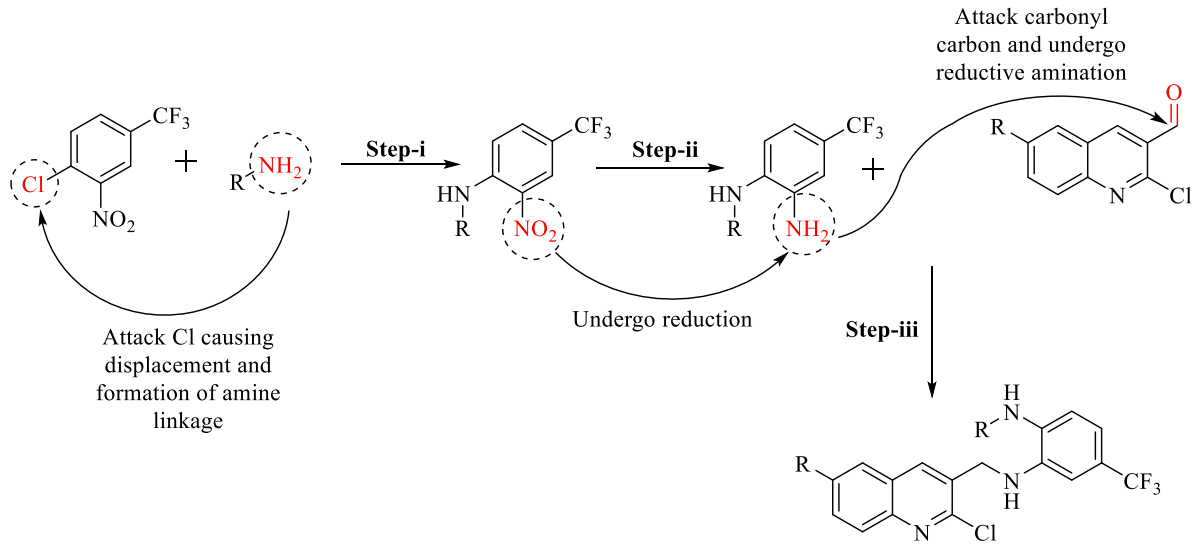
Accordingly, compounds **5i**, **5k** and **5a** were selected to determine the cytotoxicity against HepG2 and HUVEC cell lines by MTT assay method. VEGFR-2 overexpression is a hallmark of hepatocellular carcinoma and its activation also stimulates HUVEC proliferation. Therefore, these two cell lines were chosen for cytotoxicity testing. Compound **5i** (IC_{50} = 18.11 µM) demonstrated exceptional efficacy against the HepG2 cell line compared to the reference drug Lenvatinib (IC_{50} = 26.95 µM), while compound **5k** (IC_{50} = 31.69 µM) exhibited cytotoxicity comparable to the reference drug (IC_{50} = 32.29 µM) against the HUVEC cell line. A moderate cytotoxicity against the HUVEC cell line was also exhibited by compound **5i** (IC_{50} = 42.37 µM), revealing its potency against both tested cell lines.

Reaction mechanism:

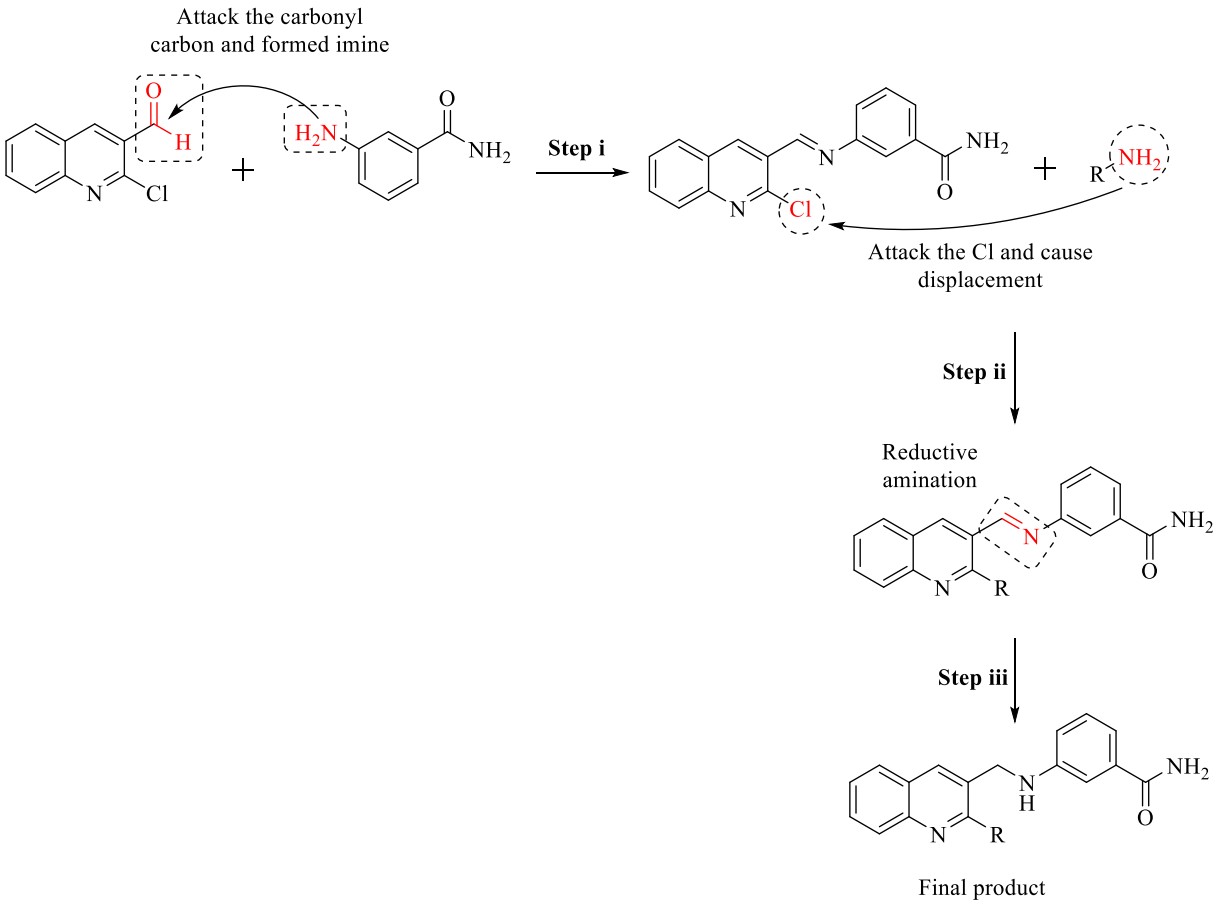
Scheme-1



Scheme-2



Scheme-3


Attack the carbonyl
carbon and formed imine
Step i
Attack the Cl and cause
displacement
Step ii
Reductive
amination
Step iii
Final product